THE ETIOLOGY OF "SYMPTOMATIC ANTHRAX" IN SWINE*

"SPECIFIC GAS-PHLEGMON OF HOGS"

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INTRODUCTION

Symptomatic anthrax, or "blackleg," has been reported and described by Mareck,[1] Born,[2] Battistini,[3] and Balas and Willenberg.[4] Arloing, Cornevin and Thomas,[5] Glässner,[6] and Wulff,[7] on the basis of transmission experiments with material from cattle, denied the existence of this disease in pigs, and questioned the inoculation experiments of Mareck. Recently, von Ratz[8] succeeded in infecting hogs with the bacillus of symptomatic anthrax, thereby reopening the question as to whether blackleg, or a similar infection, appears spontaneously in hogs. As far as I have been able to survey the available

* Received for publication July 1, 1915.

1. Monatsh. f. prakt. Tierheilkunde, 1896, 7, p. 489; 1897, 8, p. 174.
2. Veterinarius, 1897.
3. Quoted by v. Ratz, Ztschr. f. Infektions-krankh. d. Haustiere, 1913, 14, p. 1.
4. Berl. tierärztl. Wchnschr., 1908, 24, p. 734.
5. Le charbon symptomatique du boeuf, 1887.
6. Krankheiten des Schweines, 1912, p. 16.
7. Deutsch. tierärztl. Wchnschr., 1912, 20, p. 689.
8. Ztschr. f. Infektions-krankh. d. Haustiere, 1913, 14, p. 1.

literature, no cases of symptomatic anthrax in hogs have been reported in the United States. The observations and etiologic studies presented in this report are parts of our investigations dealing with the sequels of hog-cholera immunization.

HISTORY OF THE MATERIAL USED FOR THE INVESTIGATIONS

On Aug. 28, 1912, Dr. E. C. Deubler brought to the laboratory of the Pennsylvania State Livestock Sanitary Board a piece of muscle removed from the neck of a hog which had died the previous day. Clinically, the hog had shown a large, edematous swelling extending along the neck, blue-purplish discoloration of the abdomen, high temperature, distress, and diarrhea. Inasmuch as hog-cholera had existed in the piggeries on the farm from which the hog came, and all of the pigs had been immunized passively against the disease, the death of the animal was very important from a sanitary viewpoint. With the exception of the muscle lesions, no pathologic processes could be found indicative of hog-cholera. The piece of muscle, size 8 by 6 cm., was red-brownish, streaked by deep, brownish areas, very dry, friable, and spongy; the muscle fibers were separated by gas bubbles. On section, a small amount of a serous-hemorrhagic fluid could be squeezed out. The material had the characteristic acid odor of blackleg. The muscle was very light, small pieces floating on water. The microscopic examination revealed gram-positive and gram-negative organisms, with and without spores, resembling in many respects Bacillus chauvæi (Strain 1).

Dr. Deubler was instructed to make an epidemiologic investigation, and to procure a sick or a dead hog, if possible. On Sept. 9, 1912, a dead hog was sent to the laboratory and a report also submitted by Dr. Deubler to the writer, which read in part as follows:

"The piggery is located in a low, swampy place, but is constructed with concrete, so that the pigs rarely come in contact with the ground. Sixty pigs are kept in this place; no new hogs have been introduced into the pens since June last, at the time when all the hogs had been immunized against hog-cholera. The application of the serum was made subcutaneously on the inside of the thigh. The animals are fed on garbage, and drink the spring water which is collected from the hill-side near the piggery. No cases of hog-cholera have developed for the last two months.

"About August 25 one hog died; the cadaver was not examined. Another hog died on August 27, and a muscle specimen was sent to the laboratory. A third hog died on September 4, and the hog sent for autopsy died on Sept. 8, 1912.

"There is one hog which had, as had all of the others that died, a swelling on the left shoulder and neck for a few days. At the date of inspection the animal appeared normal; no swelling or rise in temperature could be detected. Altogether, four hogs have died with symptoms most suggestive of hog-cholera."

The hog sent to the laboratory for autopsy was examined about 16 hours after death, on Sept. 9, 1912.

AUTOPSY

Sow, weighing 220 pounds; good condition of nutrition; rigor mortis on limbs still present. The cadaver is bloated, the abdomen distended by gas. Along the abdomen the skin is reddish-blue and discolored, the discoloration spreading diffusely into the neck. Along the neck, and particularly near the

head, there is a diffuse, edematous-gaseous swelling. No wounds or scars can be detected in this region. From the mouth a small amount of froth is discharged. The anus is prolapsed and deep-bluish.

From an incision along the neck, a small amount of reddish fluid escapes; the subcutis is filled with small and large gas bubbles. Near the atlas, several muscle-groups are dark-reddish; on incision they crackle, are spongy and friable. A peculiar acid odor is marked. The intermuscular connective tissue is moist, and in some places slimy and yellowish. The exudate is filled with numerous gas bubbles. The peripharyngeal connective tissue is infiltrated by a serous-hemorrhagic fluid rich in gas bubbles; the sternocephalic and longus capitis muscles are deep-brown and emphysematous. The blood vessels in this region contain dark, liquid blood with numerous gas bubbles. In the left parotid region, extending along the left side of the neck, the gas formation in the intermuscular tissue is extensive; on incision a frothy, serous liquid runs off. Several portions of the brachicephalic muscle are dark and spongy. The left masseter muscle is moist and contains numerous blackish, friable, dry areas, with gas. All the portions of muscle removed have a characteristic acid odor and float on water. The retropharyngeal, submaxillary, and cervical, as well as the prescapular, lymph-nodes are enlarged, soft and juicy on section.

In the peritoneal cavity is a small amount of reddish fluid. The intestines are grayish, distended by gas. The omentum and mesentery show slight imbibition. The lymph-nodes are enlarged and soft; the structure is indistinct. The membrane of the intestinal tract is thickened in places and covered by stringy mucus. No ulcers or scars are found in the ileum or cecum. The stomach is in an advanced stage of autolysis. The liver is enlarged; the capsule, smooth; the parenchyma spongy, foamy, and light-grayish. Gas bubbles are noted beneath the capsule in small aggregations. The bile is stringy. The spleen is small, the capsule wrinkled, the pulp dry, the stroma decidedly visible. The kidneys and pelvic organs are apparently normal. The peritoneum is reddish and discolored in places.

The thoracic cavities contain about 10 c.c. of a light-reddish fluid. The lobes of both lungs are only slightly collapsed, moist, and, on section, a large amount of frothy fluid escapes. The lobes of the left lung are rich in blood, but uniformly elastic, and contain air. In the pericardiac sac are a few drops of reddish fluid. The heart is small and flabby; the ventricles contain poorly coagulated, dark blood, rich in gas bubbles; the valves are normal. The myocardium is soft and light-brownish and it crepitates on incision; there are a few gas bubbles between the muscle fibers; the intima of all the blood vessels is deep-reddish (imbibition). In the trachea is a considerable amount of blood-stained froth. The larynx is diffusely infiltrated; the tonsils are reddened and studded with gas bubbles; on incision there are numerous small plugs in the crypts. The mucous membrane of the pharynx and larynx is discolored bluish and covered with stringy mucus. In the mouth and nasal cavities is a considerable amount of blood-tinged mucus. All the mucous membranes are cyanosed.

Diagnosis.—Gas-phlegmon of pharyngeal region of the neck; acute serous pharyngitis and laryngitis; foamy liver; pulmonary edema.

BACTERIOLOGIC EXAMINATION (STRAIN 2)

Bacterioscopic Findings.—Bacilli are found in smears from the affected muscles, heart-blood, liver, tonsils, etc. The organisms resemble anthrax bacilli with rounded ends; several rods, however, are very long and are connected

in filaments. Some forms are whetstone- or lemon- or spindle-shaped, so-called "clostridium" forms; some are decidedly club-shaped. The clostridium forms and some small rods show endogenous spore formation; in the long rods the spores are in polar position. Some bacilli retain Gram's stain only in the form of granules. Some are entirely gram-negative (Fig. 1).

The bacilli in the edematous exudate are actively motile when slightly heated. The preparations from the depth of the muscle treated with Lugol's solution show that numerous organisms are brownish, either in stripes or in granules (so-called "granulose"). The findings in Specimen 1 correspond with those in Specimen 2.

The impression preparations from the surface of the liver show gram-positive rods in aggregations of long filaments and threads; spore-bearing forms are rare; gram-negative rods of the same size and thickness are numerous.

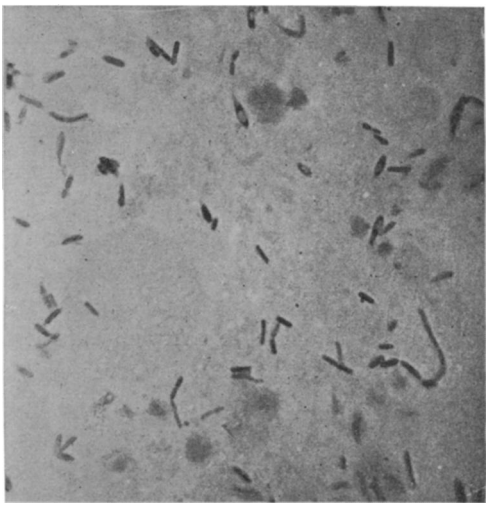

Fig. 1.—Smear from the diseased muscle (Strain 2). Carbolthionine. × 1000.

The filaments of rods are frequently in thick clusters, in which the isolated threads run parallel. Similar smears are obtained from the peritoneal lining, the serosa of the intestines, the kidneys, and the spleen.

Cultural Results.—Aerobic cultures (Strains 1 and 2) from the depths of the affected muscle portions were all sterile. The heart-blood (Strain 2) contained aerobically an organism which was identified later as B. coli. From the spleen, liver, kidneys, and tonsils were isolated streptococci and B. suipestifer.

The anaerobic cultures prepared from the muscles, heart-blood, liver, and tonsils in glucose agar, brain medium, and blood broth developed freely a gram-positive, spore-bearing rod, but no isolated colonies could be detected. The brain tubes heated for 10 minutes at 60 C. gave, in 24 hours, with marked gas production, a pure culture of the organism described. The medium remained pinkish. Plate cultures, incubated under hydrogen in Novy jars, gave

unsatisfactory results. The culture medium was beef extract agar and gelatin. Later, however, with the use of brain infusion agar, good results were secured.

Animal Inoculations.—The dried (at 37 C.) and powdered muscle substance was suspended in salt solution and inoculated into guinea-pigs, rabbits, and pigeons. All the animals died in from 16 to 36 hours. The autopsies revealed pronounced serous-hemorrhagic subcutaneous edema, the organism being found in the fluid and in aggregations of filaments on the surface of the liver.

These findings (filaments brain medium unchanged), which will be more definitely described later, led to the conclusion that the hogs were not suffering from "blackleg," but from an unknown, closely related organism.

The two specimens collected from the hogs were identical in the muscle lesion. From the history, one can conclude that 5 hogs of the piggery suffered from the same infection, to which 4 succumbed. Unfortunately, only 2 specimens could be examined, and a post-mortem made on 1 only. The muscular lesions were doubtless of the greatest interest; one regrets, however, that the history does not state to what extent the process had existed before, and to what degree it developed after, death. That a post-mandibular swelling existed is stated in the history; whether the hogs suffered from anorexia could not be determined afterwards. The anatomic alterations, consisting in serous-hemorrhagic infiltration of the subcutis, and hemorrhagic myositis, were confined to the peripharyngeal regions and the adjacent muscles. The gas formation, however, extended all along the neck to the shoulder, and in regions where no muscular changes could be seen. Along the hind legs no subcutaneous gas formation was seen. The process was apparently confined to the neck and, even if gas production occurred after death, it remained confined to the primary focus of infection.

As the cause of the lesions described, a strictly anaerobic, spore-producing bacterium was recognized, which in many respects differs from the common animal pathogenic organisms. A detailed description of the bacterium will be given later. The organisms were present in large numbers and in pure condition.

The postmortem findings suggest as portal of entry the upper digestive or respiratory tract. It is highly probable that the tonsils served as direct portal of entry, as the organisms were found there in large numbers. The inflammatory process apparently radiated from these organs. The tonsils of hogs are known to be frequently the portal of entry for bacterial infection, as for instance, in anthrax,

tuberculosis, etc. The fact that the gas formation had already badly altered the architecture of the lymph-node makes it difficult to state whether or not the plugs found in the crypts harbored the organisms and caused the infection to take place at a time when the animal proved to be otherwise lowered in vitality. No wound or other epithelial defect was noted on the head or neck which could have served as portal of entry.

The evidence and information on hand do not permit any conclusion as to when the infection occurred and how long its incubation and duration were. Some transmission experiments, described later, throw some light on this question. The number of cases indicates that a common source of infection was probably responsible. However, not every animal was susceptible to the same degree. One animal is known to have recovered. It was quite natural that the owner should have accused the hog-cholera immunization of being responsible for the death of the animals. But this idea can easily be refuted in that the time interval between immunization and the death of the hog was about 2 months. Experiments, which will be discussed later, show that the incubation time is probably less than 3 days. Cases have been known to occur in which malignant edema has developed following the careless application of serum. Usually the morbid process starts and spreads from the seat of inoculation; in our cases, the serum was applied to the inner side of the thigh.

The relation of this disease to hog-cholera is of particular importance. The clinical symptoms—discoloration of the abdomen, diarrhea, general depression, and high temperature—suggest hog-cholera and, without postmortem examination, these cases would undoubtedly have been classified with this plague of swine. The false deductions which would result from mistakes of this kind—with regard to the value of immunization against hog-cholera, etc.—are so apparent from the detailed discussion of this epidemic that the matter requires no further emphasis.

On preliminary examination, the isolated organism showed so many differences from the generally poorly described and poorly studied animal pathogenic anaerobes, that a detailed study suggested itself. The splendid methods devised by v. Hibler[9] for the study of anaerobes were followed in their essential points, particularly when details of

9. Untersuchungen über die pathogenen Anaeroben, etc., 1908.

importance concerning the points of differentiation between closely allied organisms had to be established. The results are reported in the following paragraphs.

BACTERIOLOGIC STUDY OF THE ISOLATED ANAEROBIC ORGANISM

The bacteria isolated from the lesions in the two hogs correspond in every respect; therefore, when not specially noted, the findings apply to both Strains 1 and 2.

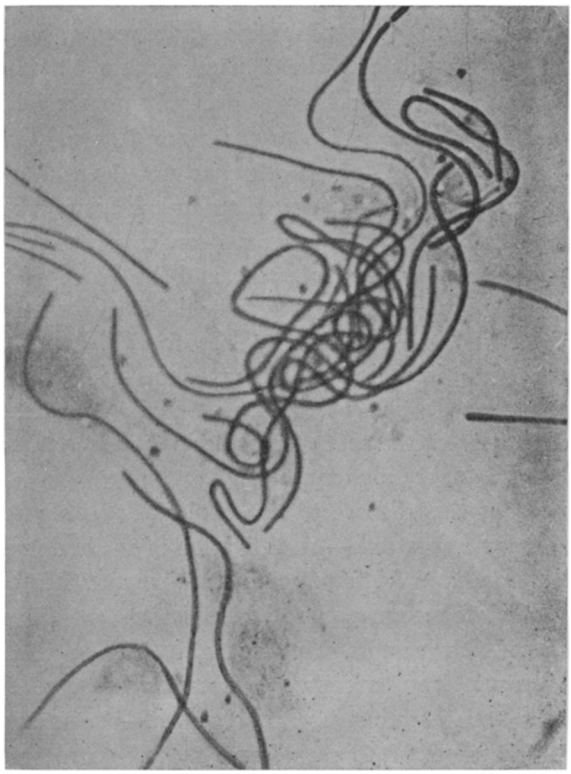

Fig. 2.—Impression preparation from the liver surface of Rat 38 (young). Carbolthionine. × 1000.

MORPHOLOGY

In general appearance the organism is a bacillus of varying size and shape. It is motile and spore-producing. The smallest rod is about 3 to 4 microns long, and 0.5 to 1.0 micron thick (unstained; Leitz's stepmicrometer); it is straight or slightly bent or curved. These forms appear in the serous exudate, in the muscle, and in the cultures. Fragments of such rods are frequently seen, resembling streptococci or other cocci-like aggregations. The longest rods

measure 13 to 15 microns, the thickness being about 0.8 micron; they are straight, bent, or wavy. The microphotograph (Fig. 2) of the organisms from the surface of the liver of a rat gives a good idea of these forms of the streptobacterium. The thickness of these filament-like rods is rarely even. In some cases they are very fine, and in others they show knob-like thickenings. The ends are mostly rounded. Some, however, show sharp-cut ends, like anthrax bacilli. The threads are sometimes segmented in small straight rods or are undivided and bent; the fine rods are rarely segmented. Some short rods also show club-shaped enlargements at one pole. By far the most interesting forms are the whetstone- or cigar-shaped, bloated or clostridium forms. They vary in size from 3 to 5 microns. Some are oval-shaped, some are pear-shaped, in most of the

Fig. 3.—Flagella stained by Zettnow's silver stain. Surface culture, 16 hours old, on glucose ascitic fluid agar (+ 0.5) in hydrogen vacuum. × 1000.

cases containing elliptical spores. These elliptical spores are of varying sizes; they are mostly 2 to 5 microns long and 1 micron thick. They are in polar or central position and very refractile. The polar spores are very frequently seen at fine rods; the organisms appear drumstick-like. The central spores usually have a small amount of protoplasm attached at each pole. The free spores usually stain slightly in the center, so that the impression is given of a ring-like arrangement in the spore.

The non-sporulated rods in animal or cultural material are actively motile. By Zettnow's silver stain, numerous peritrichal flagella can be demonstrated. From glucose ascites fluid agar slants, organisms were obtained which had from 4 to 16 flagella (Fig. 3). The motility in cultures is exceedingly variable, apparently depending on numerous factors. In glucose broth or in glucose

agar, with the customary reaction, when 24 to 48 hours old, very few motile bacilli are seen; in ascitic fluid agar with a reaction of 0.5, most of the rods develop flagella and are motile. These observations confirm the findings of v. Hibler.[10] They will be discussed more in detail later. The acid reaction, even when produced by the organisms through the fermentation of the carbohydrates, is apparently inhibitive of locomotion.

No giantwhips — as they have been described by Novy[11] for his organisms of malignant edema — could be demonstrated.

The bacilli stain readily with ordinary aniline dyes; preparations which are particularly clear are obtained with thionin. Most of the organisms stain uniformly, particularly those in the edematous fluid of guinea-pigs. In the muscular lesions of hogs, however, a large percentage of the bacteria stain faintly and irregularly. Gram's method gives positive results when 95% alcohol is used for decolorization; in all the preparations which are treated with acetone alcohol (1 : 3) mixtures, the organisms become rapidly and uniformly decolorized. In the preparations from cultures and animal material a certain percentage of bacilli always stain faintly gram-positive; some are gram-negative. The fine, filament-like rods are in most cases gram-negative. In old cultures the organisms are mostly gram-negative; some lemon-shaped forms retain the rosaniline dyes in the form of granules. The young, thick and short rods are always decidedly gram-positive.

Numerous tests to demonstrate the presence of "granulose" in the bacteria have always been successful. Dried and fixed muscle smears or preparations from glucose, starch, saccharose media, treated with weak Lugol's solution, show a large number of bacteria regularly staining brownish. Some show patchy-staining reaction; either the poles are reddish-brown or the bacillus is striped with two to three light-brownish bands. The clostridium forms frequently show marked granular "granulose-infiltration." The presence of "granulose" is constant in young cultures in the presence of carbohydrates. The extent, however, is frequently variable, and depends on factors which I have not as yet carefully studied. In any case, the preparations with Lugol's solution demonstrate clearly the described pleomorphism of the bacilli and, in our case, permitted an early diagnosis.

The various forms were distributed in the animal body and in cultures as follows: In the muscle of the hogs the short rods, the clostridium forms, and the spore-bearing rods were present in equal numbers and in regular distribution. On the surface of the liver and serous membranes of all animals examined, the long filaments of segmented or non-segmented bacilli in parallel aggregations, without spores, were the rule. In one instance, the filaments were so predominant in the peritoneal fluid that true clumps and coils of the rods were demonstrated (Fig. 2, the surface of the liver of Rat 38, Table 4). The uterine contents of Rabbit 5 were rich in whetstone- and lemon-shaped clostridium and spore-bearing forms, together with short rods. In brain and ascitic or serum media cultures the various forms, particularly spores, were always present and resembled the microscopic pictures of those made from animal material. In broth or agar cultures spores could rarely be detected; mostly medium-sized rods were present.

10. Untersuchungen über die pathogenen Anaeroben, etc., 1908, p. 145.
11. Ztschr. f. Hyg. u. Infektions-krankh., 1898, 27, p. 222 (Figs. 1, 2, 3, and 4).

CULTURAL CHARACTERISTICS

Preliminary tests had shown that the bacillus grows under anaerobic conditions. The usual media for anærobes, as blood broth (Kitt) or brain decoction (v. Hibler), gave a satisfactory growth without difficulty; but in some instances when the material was contaminated with aerobes (cocci, intestinal organisms) these media were not suitable for the biologic study of the bacillus. Pure cultures were obtained therefore by plating or deep tube methods. Most of the cultures were obtained by using material from inoculated guinea-pigs. The portions of muscle of the hogs were dried at 37 C. and kept sealed in tubes. Before inoculating the animals, small pieces were ground in a mortar to a fine powder and salt solution gradually added (about 10 c.c. to 1 gm. of substance). The suspension, heated for 30 minutes at 56-60 C., was subcutaneously introduced (on the back or in the knee-fold, by means of capillary pipet) into guinea-pigs (weight 300-400 gm.). Soon after the death of the animal the autopsy and bacteriologic examinations were carried out.

The plating method gave unsatisfactory results until I used brain or rabbit meat infusion agar containing only 0.5% glucose with the addition of sterile guinea-pig tissues. Smith,[12] Kitt,[13] and Grassberger and Schattenfroh[14] recommended, for the isolation of Bacillus chauvæi, the addition of sterile beef muscle. In the experiences of numerous workers, as well as my own, such material is rarely obtainable and even in the best organized laboratories is not always at hand. The liver, kidneys, and muscles of the guinea-pig offer a splendid substitute. Rabbit tissues are very unsatisfactory and more expensive than those of the guinea-pig when only a few pieces are necessary for the cultures. Also organs of cat and mice gave poor results. The tissues were chipped to small pieces in sterile Petri dishes and equally distributed over the bottom. The boiled and cooled agar (reaction + 0.5), inoculated with some edema or abdominal fluid or heart-blood properly diluted, was poured into the plates and quickly solidified on an iced glass plate. The plates were placed in a Novy jar, which was freed from oxygen by the combined vacuum, hydrogen-pyrogallic acid method. The hydrogen was produced in a Kipp's generator and washed according to the method of Heim.[15] In numerous tests, the recently described method of Heim,[16] of combining the pyrogallic method of Lentz with hydrogen, gave perfect results so long as the proper agar and fresh tissues were used.

To obtain isolated colonies, in many instances the deep stab cultures in agar or gelatin proved valuable. The so-called "Burri-tubes" combined with use of sterile organs need only a simple equipment and are therefore more readily at hand than the plating methods; if long tubes are used, all three dilutions can be stratified in one tube. The third dilution is placed at the bottom of the tube, in close contact with a piece of guinea-pig liver, etc.; the second and first dilutions, in turn, are poured on top, and the contents immediately solidified in ice water. For gelatin cultures, only absolutely fresh preparations of a 10% concentration with a reaction of 0.4 were found reliable. Infusions of veal, brain, or rabbit flesh were used when not otherwise mentioned. Ascitic fluid was frequently added to the agar.

12. Centralbl. f. Bakteriol., 1890, 7, p. 502.
13. Bakterienkunde und pathologische Mikroscopie, etc., 1908, p. 300.
14. Arch. f. Hyg., 1904, 48, p. 1.
15. Lehrbuch der Bakteriologie mit besonderer Berücksichtigung der Untersuchungsmethoden, Diagnostik und Immunitätslehre, 1911, p. 126.
16. Ztschr. f. d. ges. exper. Med., 1914, 3, p. 215

All the other media used for the differentiation were prepared and used as outlined by v. Hibler,[17] Foth,[18] and others. Liquid media in test-tubes were kept in Buchner's tubes or stratified with hydrocarbon oil. Flasks with broth or milk cultures were kept according to Wright's method or in Novy jars with hydrogen and vacuum. In some instances, also, brain media were inoculated, and from these, broth cultures obtained. Slant cultures were prepared on ascitic fluid agar (1% glucose) and sealed under hydrogen. On some occasions also gelatin agar (Kitt) was used for stab cultures.

Plate Cultures on Plain Agar.—By using the method of Foth, isolated colonies could be obtained on the plates. On opening the Novy jar a rancid odor escaped, resembling closely the odor of blackleg cultures. The agar was split by gas bubbles of various sizes; the water of condensation accumulated in these parts was turbid. Around the tissue pieces were seen diffusely spreading, grayish, irregular, fluffy colonies. The size of these colonies varied considerably. Some large colonies were round, smooth-edged, and finely granulated. Most of the colonies, however, were filamentous at sixty times' enlargement. From a distinctly yellowish center, filaments radiated regularly in the agar mass. When such colonies grew together, leaf-like structures could be observed. In the depth of the agar these fluffy colonies were more developed than on the surface. The outer zone of some of the colonies was more opaque than the center; the filaments were frequently toothed or club-shaped. Microscopically, motile, gram-positive rods were always found with very numerous spores, clostridium forms, and "granulose."

Streak Cultures on Agar (with Ascitic Fluid) in H Atmosphere.—A veil-like, grayish film or large, irregular colonies developed, resembling those of B. proteus. The growth was very fine and not characteristic.

Shake Cultures in 1% Glucose Agar.—In the first dilution there were abundant gas production and heavy growth, so that the water of condensation collecting between the agar fragments was very turbid and yellow-grayish. The odor was decidedly rancid. Properly isolated colonies were about the size of a large pin-head, spherical or disc-like; they were roundish, the edges very fluffy, wavy, and bush-like. When enlarged, the thick, filamentous, radiating structure of the colonies was marked.

Shake Cultures in Glucose (1%); Gelatin (12%).—In the first dilutions a uniform turbidity developed from the middle to the bottom of the tube; a few gas bubbles separated the medium; after 6 to 10 days' incubation at 22 C., the deeper layers of the medium were usually liquefied. Isolated colonies in properly prepared tubes were like cotton flakes; they were grayish, filamentous, and had irregular margins. Some were roundish with radiating inside structure and smooth margins. The liquefaction took place very slowly. The gas bubbles formed were small.

Stab Cultures in Agar and Gelatin (1% Glucose).—For only about 1.5 cm. below the surface of the medium bulk, was the growth to be noticed. It was not characteristic. The growth was filiform in agar; more rhizoid in gelatin, where liquefaction was also noted. The gas production took place very early in the growth and was extensive.

Cultures in Broth.—Only when sterile tissues had been added, was growth observed. In 24 hours, decided turbidity and pronounced gas production were noticed. Usually, in from 48 to 100 hours, the cloudiness had settled to the

17. Untersuchungen über die pathogenen Anaeroben, etc., 1908, pp. 83, 97.
18. Ztschr. f. Infektions-krankh. d. Haustiere, 1909, 6, pp. 230, 253.

bottom of the tube and there formed a thick, whitish sediment, varying in height from 0.3 to 3 cm., according to the age of the transplanted culture or to the previous animal passage. When shaken, the whitish deposit was easily suspended as granular material, which sedimented rapidly, however. These observations were particularly constant in test-tube cultures when large amounts of material were used; the growth continued for at least eight days, and the sedimentation was accordingly slow. The odor of the cultures was slightly rancid, never repulsive and of putrefactive character. The microscopic findings were interesting: Long, gram-positive, filamentous aggregations prevail in the first 16-20 hours; afterwards, short or abnormally shaped rods, whetstone-, cigar-, or pear-shaped forms with spores and "granulose," were very numerous. Sporulation and "granulose" production were well marked. In five-day-old cultures, mostly gram-negative rods and free spores, were found.

In Broth with 1% Glucose or Sodium Formate (0.3%).—Growth also took place in this medium in the absence of sterile tissues. The growth, the gas production, and the sediment were more pronounced and abundant. In Dunham's peptone solution, the growth was poor, or very slight; gas production was present.

In Glucose (1%) Sheep- or Horse-Blood Broth (Kitt).—In this excellent medium, the organisms grew very readily under aerobic conditions. After 34 hours a heavy froth arose on the fluid bulk. The fibrin clot was well filled with gas bubbles and also floated; however, no peptonization of the coagulum, even after a month, was noted. The hemoglobin was changed to a brownish, granular mass. The cultures had a strong butyric acid odor. The bacteria lost their virulence rapidly in this medium.

In Milk.—The fresh milk was kept in test-tubes, Erlenmeyer flasks, or fermentation tubes. Anaerobiosis was obtained by stratification with sterile oil, or by absorption of the oxygen, or by vacuum, etc. In all cases the changes were the same. In from 4 to 15 days the milk coagulated smoothly. A small amount of turbid whey was pressed out from the clot. After about 15 days the whey was clear and more abundant. The casein clots became attached to the walls of the tubes or flasks, and were shrunken. The curd was smooth and cheesy in a few instances only. A few holes, due to gas bubbles, were present in the tubes which had been inoculated with the infected heart-blood. The effect of the blood was probably like that of the addition of tissues. When sterile tissues had been added to the medium, quite different results were noted. The coagulation took place in from 24 to 48 hours and was associated with abundant gas production. The whey was more excessive, and had a deep yellowish tinge. The casein clots floated on it and frequently the entire coagulated mass lay directly under the cream, the whey being about two-thirds of the contents of the tubes or flasks. The clots were all bloated by gas bubbles. In from 10 to 20 days, shrinkage of the coagula also took place. The results were constant and apparently not influenced by the age of the cultures from which transplants were made or the source of material, whether animal or culture. The amount of inoculated material or culture did not alter the result as long as fresh milk, directly collected from the cow, was used. Old milk coagulated slowly, and less vigorous gas production was the rule. In 4 days about 4%, and in 30 days about 6.5%, acid was produced. The odor was always rancid, typically like that of butyric acid, never disagreeable or foul. In the tubes without tissues the odor was slight, as compared with those with tissues. The cultures were carefully studied for peptonization of the curd.

Even in cultures one and one-half years old, in which desiccation had been prevented, no liquefaction of the casein clots or flakes occurred.

In Brain Medium According to v. Hibler.—In 24 hours the semi-liquid mass was usually split by gas bubbles, which, when shaken, rose to the surface and caused frothing of the upper layers of the medium. The odor was markedly rancid, like that of butyric acid. Microscopically, there were present rods with and without spores, strongly gram-positive, containing granulose. In about 5 days the color of the brain emulsion in the deeper layers was bright-pinkish, and only a zone of about 0.8 to 1.0 cm. was grayish-brown. Over 50 tubes, inoculated with material from subcultures or directly with animal material, were kept for 1 year; no changes were noted, with the exception of a broadening of the upper grayish zone of oxidized brain material and, in some tubes, an intensification of the pink color to deep red.

TABLE 1

TESTS FOR THE FERMENTATION OF CARBOHYDRATES

Organism	Dextrose*	Lactose*	Saccharose†	Maltose*	Sorbite†
Bacillus isolated from hogs	40% 1.8% —3.2 % Heavy sediment	20% 2.6% —3.9 % Heavy sediment	Bubble (0.9% —1.2% Slight sediment	35% 2.2% —2.7% Heavy sediment	Bubble (1.0%) Heavy sediment
"Vibrion septique" de Pasteur	45% 1.8% Heavy sediment	40% 2.5% Heavy sediment	Bubble (1.5%) Slight sediment	40% 2.1% Heavy sediment	Bubble (0.7%) Slight sediment
Blackleg (Strain 2, Munich)	25% 3.5% Heavy sediment	35% 2.3% Heavy sediment	Bubble (1.3%) Slight sediment	30% 2.6% Heavy sediment	Bubble (1.3%) Slight sediment
Control	0.2 %	0.2 %	0.2%	0.15%	0.9%

* Kahlbaum. † Merck. ‡ Bausch and Lomb.
The figures give the gas and acid reactions.

The reaction was decidedly acid and remained so for the entire period of observation. Some titration tests with 1 : 100 normal sodium hydroxid gave the following results:

First day .....0.9% acid
Third day.....1.9 "
Fifth day.....2.4 "
Eighth day.....5.4 "
Eighteenth day.....6.6 "

In this medium the organism remained alive and virulent for a period of over 2 years. Subcultures could always be successfully obtained and in some instances this medium was also used to procure pure subcultures by heating 48-hour-old brain cultures (in which spores were numerous) for 30 minutes, at 80 C.

In Coagulated Blood Serum, Yolk, and Egg Albumin, and Ascitic Fluid, with and without 1% Glucose.—The organisms grew abundantly, with little or extensive gas formation, depending on the presence of fermentable carbohydrates. The coagulated protein mass, under these conditions, was split into

smaller clumps and a yellowish fluid expressed. The reaction of this fluid was always strongly acid. Peptonization of the clots has not been recorded even after a period of observation of 6 months. Nor were there present any changes in color, particularly in the yolk media. The odor resembled that of butyric acid.

In Potato, Plain, and Immersed in Sodium Carbonate Solution (0.5-2.5%.—On the ordinary potato medium no satisfactory growth could be obtained. In media containing less than 1% sodium carbonate a moderate growth was noticed in the slight gas production and the change of the medium to an acid reaction. In higher concentrations of the alkalies no growth was ever seen: apparently the spores did not germinate.

Biochemical Activities.—Indol was not formed by the organism in peptone solutions when tested by the Ehrlich -paradimethylamidobenzaldehyde method.

TABLE 1.—*Continued*
TESTS FOR THE FERMENTATION OF CARBOHYDRATES

Arabinose*	Xylose*	Rhamnose‡	Mannite†	Dulcite*	Glycerin†	Plain
None (0.8%) Slight sediment	Bubble (0.8%) Slight sediment	Bubble (0.8%) Slight sediment	Bubble (1.0%) Slight sediment	Bubble (1.0%) Slight sediment	Bubble (1.0%) Slight sediment	Bubble (1.0%) Slight sediment
Bubble (1.0%) Slight sediment	Bubble (1.4%) Slight sediment	Bubble (0.5%) Heavy sediment	Bubble (1.6%) Slight sediment	Bubble (1.1%) Poor sediment	Bubble (0.8%) Slight sediment	Bubble (0.8%) Slight sediment
Bubble (1.0%) Slight sediment	Bubble (1.5%) Sediment	Bubble (1.0%) Sediment	Bubble (1.5%) Slight sediment	Bubble (1.2%) Very slight sediment	Bubble (1.0%) Sediment	Bubble (0.9%) Slight sediment
0.2%	0.2%	0.2%	0.8%	0.8%	0.8%	0.8%

Sulphurated hydrogen was demonstrated by suspending a piece of filter paper, moistened with lead acetate solution, in the space between the cotton plug and the broth culture, which was kept in a Buchner's tube. The paper was blackened in from 48 to 72 hours. Cultures on solid agar media containing lead acetate or ferrosulphate (1:1000) grew freely, with extensive gas formation, but were never turned black; the reaction of the medium was always acid and, therefore, iron sulphid was not precipitated.

Neutral red, litmus, methylene blue, sodium indigosulphate, when added to solid or liquid media, are reduced.

Fermentation of Carbohydrates.—For the fermentation tests the method of Bahr[19] was used. Large Durham tubes each containing 1% of one of the various carbohydrates in Liebig's broth were inoculated, by means of pipets, with the sediment of the 48-hour-old cultures of the organisms to be tested. The test-tubes were kept in a Novy jar under vacuum-hydrogen and pyrogallic acid for 5 days at 37 C. Aerobic control-tests were prepared simultaneously. The tests were repeated several times. The results are tabulated in Table 1. The organism under discussion fulfills all the reactions characteristic for Group 1 in Bahr's[19] classification. It cannot be separated from the blackleg

19. Ztschr. f. Infektions-krankh. d. Haustiere, 1911, 9, p. 225.

organisms or the "vibrion septique" of Pasteur by means of the fermentation tests. The growth was extensive in the tubes where fermentation occurred; the gas and acid production fluctuated; noteworthy was the fact that small amounts of gas and acid were also produced in plain broth, probably as a result of catabolic acid on the protein substances. In 4% glucose broth, in Smith's fermentation tubes, 60-90% gas is produced. The analysis gave a gas formula of $2H/CO_2$ to $4H/CO_2$. In numerous tests these proportions were constantly found. The production of ethylalcohol, tested according to Foth,[18] was found in 2 tests to be constant.

Spore Resistance.—For the determination of spore-resistance to heat, the method of v. Hibler[20] was used. The temperature of the steam at Berkeley was between 99 and 99.5 C. Brain media in thin, long test-tubes were used for subcultures. The resistance to the heat of boiling water was very low, varying between 5 and 8 minutes. An exposure of 10 minutes to a temperature of 100 C. invariably killed the organism. The results of the various tests are tabulated in Table 2. At a temperature of 75 C., the spores remained alive

TABLE 2
RESISTANCE OF SPORES TO HEAT

Spores (Inoculated into Brain Media)	Growth (+) During Exposure to 99 - 99.5 C. From 2 to 20 Minutes								
	2 Min.	4 Min.	5 Min.	6 Min.	7 Min.	8 Min.	10 Min.	15 Min.	20 Min.
Two-day-old blood broth culture.....	+	+	+	+	—	—	—	—	—
Two-day-old beef broth culture.....	+	+	—	—	—	—	—	—	—
Twenty-four-day-old brain medium culture	+	+	+	+	—	—	—	—	—
Eight-day-old milk culture.....	+	+	+	—	—	—	—	—	—
Three-day-old brain medium culture..	+	+	+	—	—	—	—	—	—
Four-day-old brain medium culture...	+	+	+	—	—	—	—	—	—
Six-day-old brain medium culture.....	+	+	+	+	+	—	—	—	—

even after an exposure of 5 hours (longest period tested). In organ material (muscle), when kept cool, the spores remain unaltered for years (2½ years longest period tested). In broth, blood, milk, and agar cultures kept at 37 C. for longer than 4 weeks, a reduction in the number of living spores was frequently noted; in some instances, the organism even died out. Brain media (in numerous observations) were apparently more protective for the spores.

PATHOGENICITY

The pathogenicity of the bacillus was tested with animal material (muscle of the hogs) and also with pure cultures. The material inoculated was always previously sterilized from all non-spore-bearing organisms, as already indicated under the heading of cultural characteristics. The cultures varied for the different tests and are mentioned separately in Table 3. The dead animals were either examined immediately after death, or the cadavers were well kept, on ice. Each animal was carefully examined bacterologically; aerobic and anaerobic cultures were prepared.

Primary Animal Inoculations.—Aside from the inoculation of guinea-pigs for the preparation of pure cultures, rabbits, pigeons, mice, rats, and hogs were

20. Untersuchungen über die pathogenen Anaeroben, etc., 1908, p. 212.

infected with spore material from the muscles of Hogs 1 and 2. Inasmuch as the results are the same in the small animals as those obtained with pure cultures, the inoculations and the autopsies on these animals, with the exception of the hogs, are summarized in Table 3. The muscles from Hog 16037 were dried, as explained, and used for the primary inoculation experiments on the small laboratory animals, mentioned in Table 3.

These experiments were undertaken primarily with the idea of establishing a diagnosis, since, according to current opinions, all laboratory animals are very susceptible to malignant edema as compared with blackleg. The amounts chosen for the inoculations were as small as possible so that a marked contrast might be observed, particularly in rabbits. According to Table 3, the organism is pathogenic for guinea-pigs, rabbits, mice, pigeons, and rats. With the fresh material, uniformly fatal results were obtained. One year and a half later, however, the pathogenicity of the organism for rabbits and rats had noticeably diminished and it behaved in many respects like Bacillus chauvæi. The inoculation of pure cultures is therefore of more importance and the entire question is consequently best discussed in connection with the experiments along these lines.

Inasmuch as the anaerobes commonly lose their virulence quickly, it was considered advisable to conduct experiments reproducing the disease as observed in the hogs from which the material was collected, before the organism had been studied in detail and the absolute purity of the cultures ascertained. Two experiments, of which one was successful, are reported in detail. With a comparatively large dose of dried muscle a large sow was readily infected by subcutaneous application of the material behind the ear. The course of the infection resembled closely that reported from the spontaneous cases, and the post-mortem and bacterioscopic findings correspond exactly with those in Case 2. The bacillus, therefore, is pathogenic for hogs and is undoubtedly capable of producing the lesions resembling blackleg in cattle. Inoculation experiments with pure cultures were carried out about 2 years later, at a time when the strain had already lost its virulence. These tests were not as successful, as will be shown, and therefore the experiment on Hog 2 is of importance for the later discussion of the entire problem of infection with this anaerobic organism.

WHITE BARROW 16045.—Weight, 200 pounds. Inoculated on Sept. 10, 1912, with 1 c.c. of a suspension of dried muscle from Hog 1 (0.1 gm. to 10 c.c. of salt solution) subcutaneously back of the left ear. On September 11, the animal showed a hot, edematous, but not emphysematous, swelling. It appeared depressed and did not care for food. No temperature reaction was recorded. On September 14 the swelling had disappeared. The hog was killed several weeks afterwards and found to be normal.

BLACK AND WHITE SOW 16037.—Weight, 230 pounds. Inoculated, Sept. 14, 1912, with 10 c.c. of a suspension of muscle from Hog 1 (diluted as for Hog 16045) subcutaneously on the back of the left ear. The temperature curve is shown in Chart 1. On September 16, a swelling of the size of two fists, had developed behind the left ear. The animal was noticeably sick. On September 17, the swelling had spread along the submaxillary and cervical region; the hog was unable to swallow and showed also difficulties in breathing; the mucous membranes of the head were slightly cyanosed. On September 8, the hog died during the morning, about 88 hours after inoculation.

The autopsy was made 3 hours after death. The skin of the abdomen was slightly purplish. In the left auricular region, extending to the shoulder

TABLE 3

INOCULATIONS WITH HEATED, DRIED MUSCLE OF HOG 1

Animal	Material Inoculated	Seat of Inoculation	Died After	Time Elapsing Between Death and Autopsy
Guinea-pig ...	0.3 c.c. of muscle = 0.03 gm. powder	Subcutaneous	18 hr.	1½ hr.
Guinea-pig ...	0.5 c.c. of muscle = 0.03 gm. powder	Subcutaneous	14¾ hr.	10 hr.
Rabbit	0.5 c.c. of muscle = 0.5 gm. powder	Subcutaneous	.....	.....
Mice (2)	0.1 c.c. of muscle = 0.001 gm. powder	Subcutaneous	38 and 40 hr.	2½ hr.

INOCULATIONS WITH HEATED, DRIED MUSCLE MATERIAL OF HOG 16037

Guinea-pig ...	0.5 c.c. of muscle = 0.5 gm. powder	Subcutaneous	16 hr.	2 hr.
Rabbits (6)...	0.5-1.0 c.c. of muscle 1-0.5 gm. powder	Subcutaneous	14-20 hr.	½-3 hr.
Pigeons (2)...	0.5 c.c. of muscle = 0.5 gm. powder	Subcutaneous	14 hr.	Immediately examined

INOCULATIONS WITH HEATED, DRIED MUSCLE OF HOG 2

Guinea-pig ...	0.2 c.c. of muscle = 0.02 gm. powder	Subcutaneous	About 16 hr.	Immediately examined
Pigeon	0.2 c.c. of muscle = 0.02 gm. powder	Intravenous	20 hr.	1 hr.
Rat	0.5 c.c. of muscle = 0.5 gm. powder	.....	86 hr.	5 hr.
Rat	0.1 c.c. of muscle = 0.1 gm. powder	.....	Alive	.....
Rat	0.5 c.c. of muscle; 1:2 powder	Subcutaneous	Alive; discarded 6/12/14	.....
Rat	0.5 c.c. of muscle; 1:2 powder	Subcutaneous	Alive; discarded 6/12/14	.....
Rabbit	0.5 c.c. of muscle; 1:2 powder	Subcutaneous	Discarded 6/12/14	.....
Rabbit	1.5 c.c. of muscle; 1:2 powder and 4 drops of lactic acid	Subcutaneous	Discarded 6/12/14	.....
Rat	0.1 c.c. uterus contents of Rabbit T. 2	Subcutaneous	60 hr.	12 hr.
Rat	0.1 c.c. uterus contents of Rabbit T. 2	.....	54 hr.	20 hr.

TABLE 3.—*Continued*

INOCULATIONS WITH HEATED, DRIED MUSCLE OF HOG 1

Lesions	Microscopic Findings	Cultural Results
Serous, hemorrhagic edema with gas bubbles; necrosis at seat of inoculation	Short rods with spores; on peritoneum, filaments	Pure culture from heart-blood and edema
Serous, hemorrhagic edema with gas bubbles; necrosis at seat of inoculation	Short rods with spores; on peritoneum, filaments	Pure culture from heart-blood and edema
Developed a large local infiltration, but recovered completely	.....	.....
Serous, hemorrhagic edema	Short rods and numerous clostridium forms; long filaments on liver	Pure cultures from heart-blood and edema

INOCULATIONS WITH HEATED, DRIED MUSCLE MATERIAL OF HOG 16037

Serous edema in inguinal region	Short rods only; on peritoneum, filaments	Heart sterile; edema positive anaerobically
Hemorrhagic edema; fluid in peritoneum; muscles of abdomen very soft and friable; very few gas bubbles	Short, spore-bearing rods; on peritoneum, filaments	Edema and peritoneum positive; heart-blood positive in one animal
Necrosis and gas-phlegmon at seat of inoculation; hemorrhagic myositis	Distorted, spore-bearing rods; on liver surface, filaments	Muscle, anaerobically, positive; heart-blood sterile

INOCULATIONS WITH HEATED, DRIED MUSCLE OF HOG 2

Serous, hemorrhagic edema with muscular emphysema	Short rods only; filaments rare	Edema pure culture; heart sterile
Necrosis hemorrhages, and edema of pectoral muscle; peritoneal fluid	Short rods and clostridium forms	Muscle positive; heart sterile
Ulcer at seat of inoculation; serous, slightly hemorrhagic edema; no gas bubbles	Well-spored rods; on liver surface, filaments	Gluteus muscle positive
Slight edema at seat of inoculation		
Slight local edema at seat of inoculation		
Slight local edema at seat of inoculation		
Necrosis at seat of inoculation....		
Slight local edema.....		
Necrosis at seat of inoculation; slight serous edema; general congestion; duodenitis; lung edema	Spore-bearing rods in muscles and edema; on surface of liver, filaments in coils	Heart-blood and edema, anaerobically, positive
Marked serous, hemorrhagic edema; no necrosis; same as Rat 16	Spore-bearing rods in muscles and edema; on surface of liver, filaments in coils	Ditto; aerobically, heart-blood gave B. coli

blade, was a diffuse edematous swelling, which on section proved to be due to a yellowish serogelatinous infiltration of the subcutaneous and intermuscular tissues of the places mentioned. At several places a few gas bubbles were seen. In the tracheocephalic, deltoid, and trapezius muscles, several areas of the size of a small hand were dark-brown in color, and moist, and some places were emphysematous, floating in water. On incision these areas crackled, and the muscle fibers were separated by gas bubbles; the center of these muscles was patchy, yellowish and necrotic. The lymph vessels in the perimysium were very marked. Only the muscles of the left side were affected. The submaxillary and prescapular lymph-nodes were as large as plums, soft, juicy, and hyperemic on section. The trachea, larynx, and pharynx showed a slightly thickened, edematous mucous membrane which was covered with a large amount of mucus and deeply injected. In the mediastinum was a gelatinous infiltration; a few atelectatic foci in the anterior lung lobes; a few hemorrhages on the pleura.

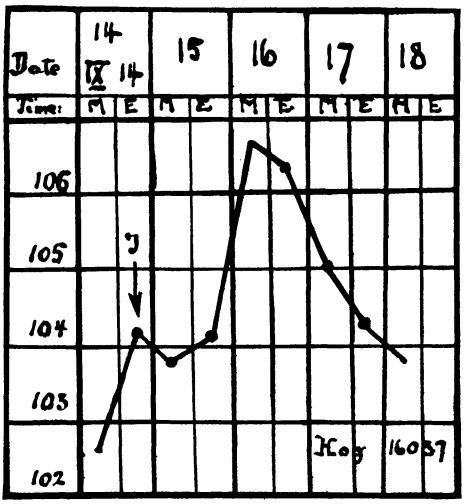

Chart 1.—Temperature curve for Hog 16045.

The fundus of the stomach was hyperemic; spleen, soft and small; liver, normal; other organs normal. The pericardiac sac contained about 15 c.c. of fluid, which was slightly turbid. The blood was well clotted; no abnormalities observed on the endocardium.

The bacteriologic examination resulted as follows—

Edema of the subcutis: Mostly long, very few short, rods in aggregations of pairs, some in filaments, distinctly gram-positive. Some were granular with spore formation. Spores were rare, also the lemon- or whetstone-shaped clostridium forms. Shadows of rods were numerous and granulose in a few clostridium forms.

Muscle juice: Clostridium forms less frequent than in spontaneous cases; endospores numerous in rods.

Pericardiac fluid: A few short rods, single, and in pairs.

Liver surface and peritoneum: No organisms noted.

Liver parenchyma: A few short rods, also a few filaments.

Cultures: (1) Heart-blood, aerobically, was sterile (enriched in broth); anaerobically, the bacillus was isolated in pure culture. (2) Edema and muscle, aerobically, gave cocci; anaerobically, the bacillus and cocci. (3) Pericardiac fluid, anaerobically, the bacillus; aerobically, sterile. Liver, aerobically, gave intestinal organisms; anaerobically, the bacillus, mixed with intestinal organisms and cocci.

Animal Inoculations with Pure Cultures.—The primary animal inoculations did not permit the injection of an exact dosage of the bacteria or spores. For a comparative study with similar organisms which have been described, only the results obtained with pure cultures are worth consideration. Most of the results are tabulated, as far as they are of interest and deal with pure infections. Some detailed reports are added for each animal, to convey to the reader an exact picture of the methods, findings, and the amount of work which were connected with these tests. The results have not been uniform, probably, for the most part, because of the gradual decrease of virulence of the strains used. Various types of cultures of varying ages were employed,—factors which also had an influence on the results and their interpretation. There is not however a different susceptibility in different species of animals, as is a well established fact for Bacillus chauvæi. Individual fluctuations, on the other hand, seem to account for some results. It will be shown that a certain amount of care has to be observed in using the results for differential diagnostic purposes.

Tests on Guinea-Pigs.—Subcutaneous inoculations only were used, the amounts of the various cultures varying from 0.1 to 0.5 c.c. In every instance, the infection was fatal, the animals dying in from 18 to 68 hours after injection. The dosage apparently had no influence on the duration of the disease; the age of the culture and the medium used were however important factors; the addition of sodium formate or the cultivation in milk apparently reduced the virulence.

The inoculated guinea-pigs became less active after varying periods of time following the injections. After 12 to 16 hours they sat uniformly quiet in the cages, their fur ruffled; at the seat of the injection, a well-defined, edematous swelling developed, which was hot, and showed a bluish discoloration in some instances. In a few cases only, crepitation was noticed. The swellings spread in the following hours and the animals grew very weak, finally lying on one side, for varying periods, before death.

The autopsy revealed the usual well-known lesions of a gas-phlegmon at the seat of inoculation. The skin was strongly infiltrated; on incision, a small amount of reddish fluid oozed out. The subcutaneous and intermuscular connective tissues along the abdomen, thorax, and inguinal and subscapular spaces were infiltrated with a serous, slightly hemorrhagic fluid; the appearance of the tissue was jelly-like and contained numerous, small gas bubbles. Between the fascia, gas bubbles were also frequently noticed. The muscles and the fascia bore, occasionally, small and large hemorrhages. At the seat of inoculation, the subcutaneous tissue was yellowish, gray, soft, friable, and necrotic.

The muscles of the abdomen were very soft and "soaked" with fluid, which frequently retained small gas bubbles. The inguinal and subscapular lymph-nodes were slightly enlarged and very soft and edematous. In the abdominal and thoracic cavities were varying amounts of fluid; in the abdomen, it was turbid and grayish in color. The serous membranes were frequently covered with small hemorrhages; the parietal peritoneum was deep-reddish, shiny, and in some places grayish. In some instances, areas with streaks or gas bubbles

were very prominent. The small intestines showed injection of the serosa, contained a bile-stained liquid, and presented catarrhal inflammation of the mucous membrane. The other organs were not constantly changed. The spleen was small. The liver was frequently dark-brownish; in a few cases, when the cadavers were not examined immediately, the organ was mottled by yellowish areas of a spongy, foamy, infarct-like character. The kidneys were deeply reddened and soft. In pregnant animals, the involvement of the uterus and fetus was very marked. The hemorrhagic-necrotic character of the uterine membrane following abortion was the most marked anatomic lesion of the abdominal cavity.

TABLE 4

EXPERIMENTS ON GUINEA-PIGS

Weight of Guinea-pigs in Grams	Material Inoculated	Seat of Inoculation	Died After	Time Elapsing Between Death and Autopsy
450	Blood broth, 48 hr. old.....	0.1 c.c. subcutaneously	18 hr.	6 hr.
375	Blood broth, 48 hr. old.....	0.1 c.c. subcutaneously	22 hr.	4. hr.
393	Blood broth, sodium formate culture, 1 day old	0.1 c.c. subcutaneously	68 hr.	4. hr.
690	Sodium formate broth, 3 days old, heated	0.2 c.c. subcutaneously	42 hr.	3½ hr.
319	Sodium formate broth, 9 days old, heated for 2 hr. at 76 C.	0.3 c.c. subcutaneously	36 hr.	8 hr.
659	Sodium formate broth, 3 days old, heated for 3 hr. at 76 C.	0.2 c.c. subcutaneously	40 hr.	6 hr.
480	Milk culture, 28 days old.....	0.5 c.c. subcutaneously	36 hr.	9 hr. at room temperature

The thoracic organs were usually not affected. The lungs were pale and perhaps a slight edema of the lung was present. The heart-blood was poorly clotted and dark.

Microscopic preparations, made from the edema, demonstrated the organisms in their various forms. The internal organs usually contained the bacteria in very small numbers, either in pairs or in aggregations of filaments. The liver and peritoneal surfaces revealed the bacteria always in aggregations of filaments and threads.

The inoculation experiment on Guinea-Pig T.-40 is reported in detail, to illustrate the foregoing statements.

GUINEA-PIG T.-40.—Weight, 659 gm. Inoculated subcutaneously, May 16, 1914, with 0.2 c.c. of 3-day-old sodium formate (0.5%) glucose (1%) broth culture heated for 3 hours at 76 C. (strain from Guinea-Pig T.-16). After 20

hours the animal was apparently much distressed; its fur was ruffled; along the abdomen and towards the thorax there was a hot, edematous, well-defined swelling. The guinea-pig died in 40 hours after inoculation.

The autopsy was made 6 hours post mortem (4 hours on ice). On shaving the hair on the abdomen from the blue-reddish hide, a reddish fluid oozed out. In the inguinal folds marked crepitation was felt. On incision a broth-like serum escaped from the fascia and intermuscular tissues. In the inguinal and subscapular spaces along the neck the edema was tinged deep-reddish. All the muscles of the abdominal wall were rich in fluid; the intermuscular connective tissue on the hind leg showed numerous petechiae. The

TABLE 4.—*Cantinued*
EXPERIMENTS ON GUINEA-PIGS

Lesions	Miscroscopic Findings	Culture Tests
Serous edema; hemorrhagic myositis of pectoral and femoral muscles; catarrhal enteritis	Short rods; clostridium forms in edema and muscles; aggregations of filaments on liver surface — typical findings	Heart-blood positive; anaerobically, sterile
Serous, hemorrhagic edema; intermuscular hemorrhage; catarrhal enteritis	Short rods; clostridium forms in edema and muscles; aggregations of filaments on liver surface — typical findings	Heart-blood positive; anaerobically, sterile
Pronounced hemorrhagic, serous, gaseous edema; hemorrhagic myositis, and necrosis at seat of inoculation	Short rods; clostridium forms; terminal spores; long rods on liver surface	Heart-blood, anaerobically, positive
Hemorrhagic, serous edema; hemorrhagic, ichorous metritis; liver infarct	Mostly long rods and a few clostridium forms and spores in uterus and liver	Uterus, anaerobically, positive
Hemorrhagic, serous, gaseous edema; foamy liver; lung edema	Short and pleomorphic rods in edema; numerous spores	Heart-blood and liver, anaerobically, positive
Typical (see separate report).....	Typical	All organs positive
Hemorrhagic, serous, gaseous edema; enteritis; lung edema; foamy liver and spleen	Rods of different sizes with and without spores; filaments on liver	Heart-blood and edema, anaerobically, positive

fascia and the finer tissue membranes were lifted up by gas bubbles. At the seat of inoculation the subcutaneous tissue and the superficial muscle layers were yellow-grayish and friable.

In the peritoneal cavity was a small amount of turbid, reddish fluid. The abdominal walls were moist; the parietal peritoneum was bright red and retained gas bubbles. The small intestines were distended by gas; the serosa was injected; the contents in the duodenum were liquid, and yellowish in color. The stomach was empty; in the fundus, numerous hemorrhages and superficial erosions, and beginning auto-digestion. The liver was light-brownish; some areas were anemic and compressed by gas bubbles; on section the color was mottled. The spleen was small, but dark. The kidneys were brownish and showed localized injection. Bilateral pregnancy existed; the uterine fluid was reddish; the placenta, hemorrhagic.

In the thoracic cavity, a few drops of fluid were present; the lungs were pale but edematous; a few petechiae on the tracheal mucous membrane; the heart was light-brownish, and the blood was well coagulated in both ventricles.

The bacteriologic examination resulted as follows—

Edema of subcutis and uterus: Gram-positive rods; cigar-shaped and clostridium forms rare; a few spore-bearing rods; long rods very thin, gram-negative. Granulose formation typical in a few clostridium forms.

Muscle juice: Short, gram-positive rods, a very few spore-bearing.

Peritoneal lining: Long filaments of rods; no spores.

Liver: Long rods and aggregations in pairs.

Kidney, spleen, and heart-blood: A few gram-positive rods, some in aggregations of filaments.

Cultures: Anaerobically, the edema, the organs, and the heart-blood were positive; aerobically, they were sterile.

TABLE 5

EXPERIMENTS ON RABBITS

Weight of Rabbit	Material Inoculated	Seat of Inoculation	Died After	Time Elapsing Between Death and Autopsy
2350	Blood broth culture, 48 hr. old....	1 c.c. subcutaneously on back	.....	.....
2200	Blood broth culture, 48 hr. old....	0.5 c.c. intravenously	10 min.	Immediately examined
2670	Blood broth culture, 12 days old..	0.5 c.c. intravenously	About 88 hr.	7 hr.
1950	Blood broth culture, 4 days old...	1.5 c.c.	.....	
2300	Blood broth culture, 0.5, 1.0, 2.0, respectively	Intravenously	14 hr.	8 hr.

In Table 4, the experiments on guinea-pigs with pure cultures are summarized.

Tests on Rabbits.—Experiments on rabbits with pure cultures are of the greatest interest inasmuch as they frequently permit a diagnosis in one or the other direction. Most of the workers with anaerobes have an idea that the rabbit is relatively resistant to an infection with Bacillus chauvæi. On the other hand, it is very susceptible to organisms belonging to the malignant edema group of bacteria.

The tests which the writer has been able to carry out apparently did not entirely support this view; the first inoculations showed a marked susceptibility on the part of the rabbit to the organism isolated from the hogs. Subcutaneous inoculation with cultures failed to verify the first results. Only one explanation can be offered for this discrepancy, namely, the loss in virulence. Most of the cultures used for these tests were prepared with muscle material already 1½ years old, and it is a known fact that such anaerobes lose their virulence gradually, so that the difference in pathogenicity for certain species

of animals becomes more and more pronounced. On the other hand, some of the results which are tabulated in Table 5 bear out the findings of Ghon and Sachs, who first described the organism, found in this instance in blackleg of hogs.

Subcutaneous inoculations in small doses caused no reaction whatever; in large doses, a well-defined, edematous swelling developed, which resulted in necrosis of the skin and ulcer formations with much retarded healing. The injected organisms could be isolated from the necrotic tissue.

Intravenous inoculations were fatal in two instances. One experiment is not conclusive, however, as the rabbit had already received several other injections and probably succumbed to an anaphylactic shock caused by the foreign protein introduced with the blood cultures.

The only successful experiment is reported herewith, in detail. It shows that the septicemic character of the infection caused a picture very different

TABLE 5.—*Continued*
EXPERIMENTS ON RABBITS

Lesions	Microscopic Findings	Culture Tests
Healed ulcer on the back. Discarded 6/15/14		
Negative; probably embolic.....		
Sero-fibrinous pleurisy and pericarditis; hemorrhagic-ichorous metritis; spleen tumor; kidney infarcts; myositis; local edema. Discarded 6/15/14	Long and short rods with spore formation; typical	Heart-blood, uterus, etc., anaerobically, positive
Lung emphysema; hemorrhagic metritis; anaphylaxis; pregnancy	Short rods and clostridium forms in uterus; long rods in heart-blood	Heart-blood and uterus, anaerobically, positive

from that resulting from subcutaneous injection; namely, sero-fibrinous exudates on all serous membranes, and localization of the gas-phlegmon in the muscles and the uterus. The picture is strikingly similar to the one seen occasionally in infections with Bacillus chauvæi in cattle. The microscopic examination revealed a picture different from the usual, in the most remarkable richness in shape and varieties of sporulated and non-sporulated, free granulose-bearing forms in the uterus of the rabbit.

RABBIT J.-2.—Weight 2,400 grams. Inoculated intravenously with 0.5 c.c. blood broth culture (12-day-old strain from Hog 2). Died about 88 hours after inoculation. The autopsy was made 7 hours post mortem. The animal was bloated, with slight rigor mortis. Subcutaneous tissue, moist; blood vessels well injected; mammary glands diffusely dark-red, showing numerous small and large interglandular hemorrhages.

In the abdominal cavity, a considerable amount of reddish fluid was present. The serous lining of cecum and colon was covered with petechiae; in the peritoneal lining in the inguinal region, diffuse hemorrhages, suffusions, and petechiae.

The uterus and both horns were brownish-red and distended by gas. On opening there were in the left horn two placentas with black-brownish fetal envelopes, surrounded by a frothy, purulent exudate; in the right horn, one placenta, black in color. The uterine membrane was diffusely reddened, covered by pus and fibrin clumps; in some areas, it was lifted by a few large gas bubbles. The external os was smeared with clumps of purulent-necrotic material. Vaginal membrane showed pus and fibrin flakes and necrotic tissue; longitudinal streaks of hemorrhages very prominent. The serosa of the urinary bladder was reddened, showing some petechiae.

The spleen was enlarged; edges rounded; pulp, black and soft. Liver was slightly enlarged, pale in color and soft; bile, liquid and light-greenish. Right kidney was light-brownish with a yellowish area, the size of a pea, surrounded by a bright-red zone of demarkation; medulla, purplish in color. Left kidney, the same, with two well-demarkated, anemic infarcts. Serosa of the duodenum, covered with petechiae; contents, liquid and greenish. Colon contained semisolid contents; mucous membrane slightly swollen.

The right pleural cavity contained about 20 c.c. of a serous-hemorrhagic fluid. Costal pleura, covered with petechiae along ribs. Anterior right and left lobe loosely attached to pericardiac sac by fresh fibrin threads. Pericardium contained a few drops of fluid; it was covered by fibrin in streaks. Both ventricles, at maximal distention; blood, well-coagulated; in left ventricle, a few hemorrhages beneath the valves. Myocardium, soft and pale. All lobes of the lungs mottled; foam present in trachea and bronchi; mucous membrane injected, showing multiple petechiae and suffusions. Thymus dark-grayish, covered with petechiae. Gastrocnemic muscle, spongy, dark in color, and dry. Popliteal lymph-nodes, enlarged, moist, and hemorrhagic on section.

The bacteriologic examination resulted as follows—

Hanging drop of uterus exudate: Very long rods; oval-shaped forms with and without spores. Short forms very actively motile, some showing slight "granulose" formation; most rods gram-positive; spores confined to clostridium forms.

Pericardiac and pleural exudates: Long and short gram-positive rods; a few spore-bearing, clostridium forms.

Popliteal lymph-nodes: Short, gram-positive rods in pairs; no spore-bearing, spindle-shaped forms.

Liver: Short and long rods on the surface; in parenchyma, mostly spore-bearing, clostridium forms.

Heart-blood: Short, gram-positive rods; no spore-bearing rods.

Cultures: (1) Anaerobically, the spleen, uterus, pericardiac exudate, pleural fibrin, and heart-blood yielded heavy growth; (2) aerobically, heart-blood, uterus, pleural exudate, and spleen were sterile.

Tests on White Rats.—The transmission experiment with muscle material revealed the fact that white rats are very resistant to infection with the bacillus isolated from hogs. With pure cultures the results were conclusive. Small doses applied subcutaneously or intramuscularly caused the local reactions already described for the rabbits, but in no instance did death result from the infection. An intramuscular injection of a large dose proved fatal; the young rat succumbed to the infection in 16 hours. The subcutaneous edema was slight; the hemorrhagic, spongy character of the muscle resembled greatly the lesions seen in the hog; a catarrhal inflammation of the small intestines was also present. The microscopic findings were as usual.

Tests on White Mice.—In 2 experiments the bacillus proved to be pathogenic for white mice. Very small doses, by subcutaneous application, caused

the death of the animals in from 18 to 20 hours. The anatomic lesions consisted in a slight serous infiltration of the subcutis, marked enteritis, and slight degenerative changes of the parenchymatous organs. The bacteria were rare in the internal organs. The writer has the impression that the death and the lesions in the animals were mostly due to the toxins which were inoculated with the broth cultures. Further experiments, to prove this statement, will be reported farther on in this paper.

Tests on a Pigeon and a Chicken.—To confirm the results obtained with muscle material, one pigeon was inoculated intramuscularly; the injection of 1 c.c. caused death in about 18 hours. The changes, in the form of a marked gaseous myositis, are described in detail in the appended report.

PIGEON A.—Inoculated, intramuscularly in right pectoral muscle with 1 c.c. of a 48-hour-old blood broth culture. Died in about 28 hours. Examined 2 hours post mortem: The right pectoral region was puffed and crepitant, discolored bluish. On incision, the subcutis and muscle were found infiltrated by a serous fluid mixed with gas bubbles. The muscles were separated by a gelatinous, gaseous mass on the fascia. The muscle, on section along the needle tract, throughout a circular area of about 2 c.c. was hemorrhagic, very friable and moist; several bundles were yellowish and necrotic, diffusely dark. The entire muscle tissue was spongy and separated by gas bubbles. The duodenal loop was deeply injected, the contents blood-tinged, liquid; the mucous membrane swollen; spleen small; liver dark-brownish and mottled in areas. A slight edema of the lung was present.

The bacteriologic examination resulted as follows—

Muscle: Numerous gram-positive rods; clostridium and cigar-shaped forms, common; sporulation, very extensive; also a few free spores. "Granulose" present in oval forms.

Liver and intestinal surface: Short and long rods, in pairs, and aggregations of filaments; no sporulated forms.

Heart-blood: Numerous gram-positive, short, plump rods; no spores.

Cultures: Heart-blood, muscles, liver, and kidneys, anaerobically, positive; aerobically, heart-blood and muscle, sterile. Kidneys show cocci.

A chicken inoculated with the same amount did not develop the slightest local reaction, nor did it succumb to an infection.

Tests on Hogs.—The writer has already discussed the successful reproduction of the anatomic picture of the lesions found in the hogs that had apparently succumbed to a blackleg-like infection, by subcutaneous injections of original muscle material. The experiment did not permit of any conclusion, however, nor did it furnish evidence for the conception advanced that the bacillus causing the retropharyngeal gas-phlegmon had entered by the tonsils or the upper digestive tract. The tests with pure cultures had therefore only the object of reproducing, if possible, the clinical picture and the anatomic lesions by direct intratonsillary injections. Unfortunately, these tests could only be carried out with material which had already been kept for over a year and which had lost its virulence, as was shown elsewhere. The results are not absolutely conclusive.

HOG 1256.—Weight, 8,880 grams. Inoculated, June 19, 1914, with 1.5 c.c. blood broth culture (40 hours old) in the left tonsil. The tonsil was slightly infiltrated after the injection. The temperature reaction of the hog is shown in Chart 2.

June 20. A tender, large edema was present in the submaxillary space, extending along the left parotid region and spreading over the left side of the neck. The animal was less active.

June 21. The swelling had not spread; the hog was lifeless, depressed, refused to eat.

June 22. Edema smaller; the hog was eating a small amount.

June 23. Edema nearly disappeared; the animal active and eating well.

June 26. Edema had disappeared; the hog apparently well.

July 8. A large abscess in the left retropharyngeal region had ruptured overnight. The creamy pus contained clumps of necrotic tissue. The abscess cavity communicated with the left tonsil by a small, fistulous canal. The pus contained cocci, B. suipestifer, and spore-bearing organisms resembling the one inoculated. A pure culture could not be obtained. On left tonsil, a small necrotic area was present.

The inoculation of 1.5 c.c. of a broth culture into a tonsil reproduced the clinical picture characteristic of the hogs which were infected during the out-

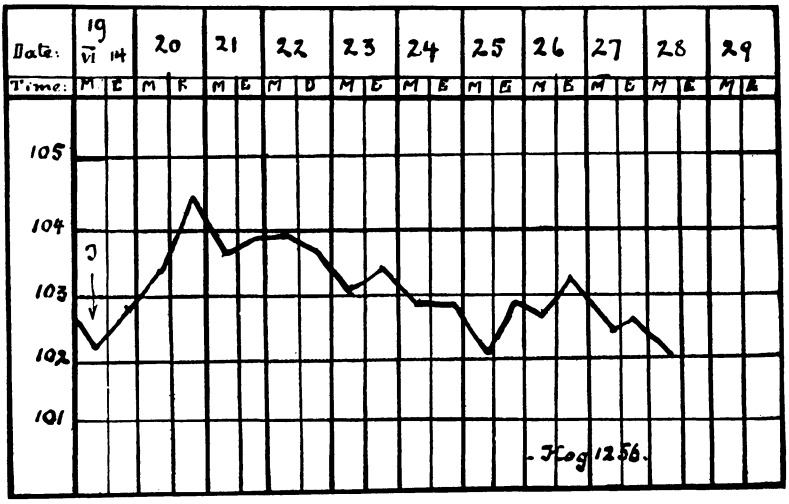

Chart 2.—Temperature curve for Hog 1256.

break, as described. Doubtless the amount inoculated and the virulence were responsible for the fact that the hog did not succumb to the infection. The pharyngeal edema resulted in necrosis of the mucous membrane, ulcer formation in the tonsils, and secondary infection with pyogenic organisms, which gave rise to an abscess in the retropharyngeal region. Microscopically, the inoculated organism was present; a pure culture, however, could not be obtained. Another attempt was made with a large amount of culture.

Hog 4.—Weight, 4,280 grams. Inoculated under ether narcosis, July 8, 1914. in both tonsils, with 3 c.c. to each of a blood broth culture (15 days old).

July 9. The animal was depressed, refused to eat. Temperature 39.10 C.

July 10. Temperature 40 C. A soft, tender swelling in submaxillary space.

July 11. Temperature 39.5 C. The hog breathing with difficulty, with a snoring sound on inspiration and expiration. The mucous membranes slightly cyanosed. The hog refused to eat and was apparently very sick.

July 12 and 13. The swelling was less marked, the breathing less difficult; the hog was eating a small amount.

July 14 to 20. No clinical observations made.

July 21. Hog found dead.

Autopsy: Rigor mortis, slight. Weight, 3,650 grams. The lymph-nodes of the groin were enlarged, appearing hyperemic; the axillary lymph-nodes not enlarged. The regional lymph-nodes in the sublingual spaces were enlarged, the ones on the right being the most marked. These felt firm; on section they appeared whitish; no evidence of suppuration. The submaxillary glands appeared normal. Those on the left-side were enlarged; appeared light on section. The left submaxillary lymph-node appeared normal. The tonsils were represented by a mass of necrotic tissue with two tubular fistulas, one of which communicated with the esophagus. One fistulous tract also extended anteriorly beneath the tongue. The tongue appeared normal. The entire pillars of the fauces presented a necrotic area, about 4 mm. in diameter, yellow in color.

The lymph-nodes of the anterior mediastinum were enlarged, firm, and, on section, white in color, tho presenting a number of small, white-yellowish areas, suggesting necrosis. The peribronchial lymph-nodes were enlarged but showed no caseation. The right lung was adherent to the pleura by numerous recent strips of fibrin. The right lung appeared rather dark red in color. The pleural surface presented numerous white areas, for the most part, circular or ovoid in shape, about 0.5 cm. in diameter. These did not extend into the parenchyma of the lung. On section, the lung exuded a bloody fluid; no areas of consolidation. The left lung was normal in color, with consolidation throughout; numerous small, white areas on surface. The inferior edge of the pleura of the lower lobe presented a mass of fibrin. On section the exudate was bloody in nature. The cut surface presented no pneumonic areas. The heart was normal in size. The pericardium was firmly adherent to the heart. The myocardium appeared pale and anemic.

The abdominal cavity had no excessive fluid. The spleen was slightly enlarged and dark in color, on section showing an increase of connective tissue. The surface presented a number of white, circumscribed areas about 1 cm. in diameter, which did not, however, extend into the substance. On section the surface appeared rather pale, otherwise negative. The kidneys were normal in size but pale; capsule stripped easily; on section appeared anemic, otherwise negative. Both had the same appearance. The mesenteric lymph-nodes were enlarged, on section showing white areas. The intestines contained a very small amount of material.

Diagnosis.—Necrosis of both tonsils; suppuration and necrosis of the pillars of the fauces; lymphadenitis of the retropharyngeal and submaxillary lymph-nodes; fibrinous pleurisy and aspiration-pneumonia of the lobes of the left and right lungs; septicemia.

A very superficial bacteriologic examination revealed B. coli, B. suipestifer, and bipolar organisms in the heart blood; B. necrophorus, and probably the injected organism, and bipolar organisms in tonsils and lung.

The clinical picture was aggravated; the temperature curve was typical, but a detailed study of the symptoms was not made. Doubtless the animal succumbed, not to the infection with the bacillus, but to a septicemia which followed on aspiration-pneumonia. The seat of injection was so severely necrotic and so invaded by secondary organisms that the pneumonia was quite in accordance.

Both experiments on hogs demonstrated that the bacillus isolated from the muscle of the hogs is capable of producing, after intratonsillary injections, a febrile reaction, and a marked edema in the submaxillary and retropharyngeal spaces, which causes disturbances in respiration and swallowing of food. The symptoms are similar to those seen in one of the hogs spontaneously infected. The naturally infected hogs possessed a reduced vitality on account of having been subjected to the hog-cholera immunization, a point which does not enter into the described experiments; furthermore, the virulence and the age of the cultures used are very important. Considering all these points and the transmission experiments with muscle material, we can accept the results as evidence that the bacillus is pathogenic for hogs and most probably enters the system by way of the tonsils or upper digestive tract.

Discussion of pathogenicity tests on animals.—In old text-books and even in some scientific articles, the animal experiment is heralded as one of the best tests of whether an anaerobic organism producing a blackleg-like affection in animals, is B. chauvæi, or not. The susceptibility of the various animals is used for the differential diagnosis. Pigeons are absolutely immune to the bacillus of symptomatic anthrax. Lehmann and Neumann,[21] Günther,[22] Gutzeit,[23] and others consider rabbits, rats, and pigeons immune to blackleg infections. Nocard and Roux,[24] Leclainche and Vallée,[25] Kitt,[26] and Foth[18] attribute to rabbits a certain degree of susceptibility. These statements have warranted numerous experiments with many blackleg strains in comparison with the bacillus isolated from the hogs. Some of these tests have been reported elsewhere. The results have not been uniform, neither with the muscle material from blackleg cases nor with our hog culture. The high resistance of rabbits against blackleg material is only relative and doubtless more marked with muscle material than with pure cultures. Exceptions have been noticed however, and it was particularly striking that the bacillus under discussion behaved like one of those rabbit-pathogenic blackleg strains. As in blackleg, large doses only are fatal for rabbits and rats, as is seen from the series of inoculation experiments, reported in Table 6. Any conclusions drawn from

21. Atlas & Grundriss der Bakteriologie, 1912, II, p. 497.
22. Lehrb. der Bacteriologie, 1906, p. 433.
23. Centralbl. f. Bakteriol., R., 1904, 34, p. 195.
24. Ann. d. l'Inst. Pasteur, 1900, 14, p. 596.
25. Ibid., p. 207.
26. Kolle and Wassermann's Handb. d. pathogen. Microorganismen, 1912, 4, p. 820.

such tests would be entirely erroneous and useless without the necessary microscopic examination and anatomic studies.

Von Hibler[27] demands, as prerequisites for pathogenicity tests and the use of the same for differential decisions, that the organisms to be tested should not be in a state of attenuation or atavistic metamorphosis. No doubt every careful bacteriologist will aim to have the organisms which he is testing restored to their original virulence and selective pathogenicity. The means suggested by v. Hibler for achieving such a condition, namely, cultivation in brain, serum, or tissue media, or the procedure of mixed infection, are all satisfactory for some cases, but in my study they have not proved to be very suc-

TABLE 6
THE INFLUENCE OF THE DOSAGE OF CULTURE ON THE MORTALITY

Animal	1.0 c.c.	0.5 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.05 c.c.
Guinea-pig	1 injected Died	1 injected Died	1 injected Died	2 injected Died	4 injected Died	
Rabbit.....	2 injected 1 died	3 injected 2 died				
Rat.....	3 injected 1 died	.....	.....	1 injected Lived	1 injected Lived (Died from intercurrent disease)	
Mouse.....	.....	.....	.....	.....	1 injected Died	1 injected Died
Pigeon.....	1 injected Died					

cessful. The virulence once lost could not be restored, and therefore the results on animals are not particularly useful for diagnostic purposes.

On the other hand, the microscopic findings on guinea-pigs, rats, etc., permitted conclusive decisions. It has been shown by Leclainche and Vallée,[25] Foth,[28] and others that B. chauvæi never produces aggregations of filaments on the surfaces of the liver and the peritoneum, a characteristic in common for many organisms belonging to the malignant edema group. The bacillus isolated from hogs is therefore not B. chauvæi, but a representative of the malignant edema group.

The character of the lesions in the injected animals, particularly the serous-exudative infiltration of subcutis, is worth consideration

27. Untersuchungen über die pathogenen Anaeroben, etc., 1908, p. 239.
28. Ztschr. f. Infektions-krankh. d. Haustiere, 1909, 6, p. 221; 1910, 8, p. 156.

when studying such closely allied organisms. In blackleg infections, the exudate is always markedly hemorrhagic; in malignant edema infections, however, it is only serous, with occasional hemorrhages. The experiments described show that the infiltration was mostly of the serous type. Different results, when they were obtained, are to be explained, I think, by the fact that large doses, and probably toxins also, were inoculated. These toxins produce hemorrhages with comparative ease, as will be shown later. The local necrosis and the production of gas are inconstant and depend on factors which are not easily controlled.

From the animal experiments one can therefore conclude that the bacillus isolated from hogs is a representative of the malignant edema group; in many respects, however, it is closely related to the B. chauvæi.

IMMUNOLOGIC TESTS

Leclainche and Vallée,[25] Foth,[18] and Markoff[29] suggested the agglutination tests as the ideal method for the identification of anaerobes. The use of guinea-pigs immunized against an identified strain of the suspected organism to be tested, has also been suggested. The latter method, however, is exceedingly delicate and only promising when sufficiently controlled. Either actively or passively immunized guinea-pigs can be used. The writer has not been very successful with the active immunization, and will report on these tests later, when they have been extended along different lines. The agglutination and serum immunization gave striking results, and are here reported more fully:

Agglutination Tests.—The preparation of immune sera offered some difficulties; some of the rabbits lost rapidly in weight and developed subcutaneous abscesses containing B. cuniculisepticus. Twenty-four-hour-old, or even twelve-day-old broth or blood-broth cultures, poor in toxins, were injected intravenously at intervals of 7 days. This method of immunization produced, after 4 injections, a very potent serum, as here reported.

RABBIT T.-3.—Immunized with the bacillus of hog disease as follows:

May 18.—0.5 c.c. blood-culture, 24 hours old, intravenously. Weight, 3,100 gm.

May 25.—0.5 c.c. blood-culture, 12 days old (a toxic strain), intravenously. Weight, 3,000 gm.

June 4.—1.0 c.c. broth culture, 6 days old (a toxic strain), intravenously. Weight, 3,200 gm. Bled; agglutinates 1:4,000.

June 15.—3.0 c.c. blood-broth, 8 days old, intravenously. Weight, 3,400 gm.

June 23.—Bled to death. Weight, 2,650 gm. Agglutinates 1:20,000 in 20 minutes.

For comparison, other sera were produced by immunizing rabbits with B. chauvæi, "vibrion septique," and malignant edema organisms obtained from the American Museum of Natural History in New York.

The bacterial suspensions were either 24-hour-old glucose broth cultures or salt suspensions of centrifugated and washed blood cultures. In some

29. Centralbl. f. Bakteriol, I, O., 1911, 60, p. 211.

instances, also, surface cultures on serum agar were suspended in carbolized salt solution. For the tests, fresh cultures only were used; they were shaken, filtered, and standardized to a density equal to a 24-hour-old typhoid broth culture. Dilutions of the serum were prepared as usual by adding 0.1 to 0.0001 c.c., etc., of serum to 1 c.c. of bacterial suspension. The results are shown in Table 7.

This test showed conclusively and more precisely than any other test that the bacillus isolated from hogs is not a B. chauvæi nor a malignant edema bacillus (Koch). The agglutination was in every test marked; flocculation occurred in from 5 to 10 minutes and was usually completed in 1 hour. The fact that the "vibrion septique" obtained from the Institut Pasteur was agglutinated nearly to the titer limit, appeared first as evidence that the organism isolated from the

TABLE 7

SERUM OF RABBIT IMMUNIZED AGAINST THE HOG BACILLUS

Strain of Organism	Dilution of Serum Causing Complete Agglutination After 24 Hours
Bacillus isolated from hogs	1 : 20000
"Vibrion septique" de Pasteur	1 : 10000
B. chauvæi (Inst. Pasteur)	1 : 60
B. chauvæi (Kitt, Munich)	1 : 100
B. chauvæi (California I)	1 : 40
B. chauvæi (California II)	1 : 80
B. chauvæi (California III)	1 : 40
B. chauvæi (Mohler IV)	1 : 20
B. œdematis maligni. (Koch I)	1 : 10
B. œdematis maligni. (Koch II)	—
Controls	—

hogs was a "vibrion septique." Comparative tests, which will be reported and discussed elsewhere, proved the identity of the two organisms. The importance of this fact will be the subject of further consideration.

For the identification of anaerobes, the agglutination test seems to be very reliable. I have therefore undertaken a more detailed study of the whole problem and hope to report further on this subject. The complement-fixation test will also be considered at the same time.

Serum-Immunization of Guinea-Pigs.—Foth,[28] Markoff,[29] and Wulff[30] particularly, demonstrated that old guinea-pigs can be immunized passively by using large amounts of immune serum and that such animals can be used for differential purposes. A few experiments along these lines were carried out further to prove the conception that the bacillus isolated from the hogs is not a B. chauvæi, but an organism identical probably with the "vibrion septique."

30. Deutsch. tierarztl. Wchnschr., 1912, 20, p. 611.

The guinea-pigs were injected with serum and simultaneously with the culture (24-hour blood broth) to be tested. Some of the results are tabulated in Table 8.

These experiments demonstrated that the rabbit immune serum obtained with the hog bacillus, protected the guinea-pigs against the bacillus and against the "vibrion septique" of the Institut Pasteur. Animals immunized against the hog bacillus were tested 17 days afterwards and proved to be immune only to the "vibrion septique," but not to any blackleg strain.

The conclusion can therefore be reached that serologically the bacillus isolated is related only to the "vibrion septique," and is positively not a B. chauvæi.

TOXIN PRODUCTION BY THE BACILLUS

Sterile filtrates of 4- to 10-day-old glucose broth cultures, prepared and treated in the essential points like the blackleg toxin of Grassberger and Schattenfroh,[31] contained toxins which proved fatal to rabbits, guinea-pigs, and white mice by intravenous or intraperitoneal inoculations. The very few tests carried out do not permit of final conclusions, and a detailed report is postponed until some essentially important and necessary comparative tests have been completed.

GENERAL DISCUSSION WITH REFERENCE TO THE IDENTIFICATION OF THE BACILLUS CAUSING A BLACKLEG-LIKE DISEASE IN SWINE

From the muscles of two hogs which had succumbed to a disease resembling blackleg in the gaseous, hemorrhagic condition of the muscles, an anaerobic bacillus was isolated. A detailed bacteriologic examination was carried out with a view to throwing more light on the question of so-called "blackleg" in swine and of determining whether such infections—when most similar anatomically—are actually due to B. chauvæi. The problem undoubtedly presents numerous questions concerning anaerobic organisms in general. Therefore, it is not surprising that the study of the bacillus developed into a comparison of the well-known pathogenic anaerobes of the blackleg-gasphlegmon malignant edema group. Even tho the report does not specifically mention all these detailed investigations, nevertheless they had to be carried out, and the entire aspect of the study was therefore broadened.

It is quite evident that the organism isolated has certain features in common with the malignant edema bacillus, particularly with the

31. Handbuch der Technik und Methodik der Immunitätsforschung, 1907-1908, 1, p. 161.

TABLE 8

EFFECT OF ANTIHOG-DISEASE SERUM ON GUINEA-PIGS

Weight of Guinea-pigs in Grams	Date of Inoculation	Amount of Serum in c.c.	Test Cultures	Amount of Culture in c.c.	Result	Control Tests on	Culture	Result
720	May 29, 1914	5.0	Hog bacillus	0.25	.....	June 16, 1914	Blackleg (Munich) 0.1	Died in 22 hr.
735	May 29, 1914	...	Hog bacillus	0.1	Died in 32 hr.	.....	.....	.....
520	May 29, 1914	5.0	Hog bacillus	0.2	.....	June 16, 1914	"Vibrion septique" 0.2	Alive
664	May 29, 1914	5.0	Hog bacillus	0.25	.....	June 16, 1914	Hog bacillus 0.1	Alive
600	May 29, 1914	4.0	"Vibrion septique"	0.25	.....	June 16, 1914	Blackleg (Inst. Pasteur) 0.2	Dead in 18 hr.
470	May 29, 1914	4.0	"Vibrion septique"	0.25	.....	June 16, 1914	"Vibrion septique"	Alive
676	June 16, 1914	4.0	Blackleg (Calif.)	0.2	Died in 24 hr.	.....	.....	.....
538	June 16, 1914	5.0	Blackleg (Munich)	0.25	Died in 18 hr.	.....	.....	.....
821	June 16, 1914	6.0	Blackleg (Pasteur)	0.25	Died in 30 hr.	.....	.....	.....

"vibrion septique" of the Institut Pasteur, of which an original culture served as control for my experiments. One would be justified in diagnosing the isolated organism as a "vibrion septique," and, inasmuch as Koch[32] considered this organism identical with the bacillus which he and Gaffky[33] had obtained from soil, the bacillus of the hog muscle would be a bacillus of malignant edema. The latter deduction is proved wrong however by several differences in my observations from the descriptions given by Koch and others.

The original descriptions[34] of the "vibrion septique" and B. œdematis maligni are so incomplete that, based on the meager data, they could not be considered, today, as being identical, since the perfected bacteriologic technic has shown that anaerobiosis, motility, sporulation, microscopic appearance, and animal pathogenicity are insufficient criteria for an identification. All investigators (Liborious,[35] Jensen and Sand,[36] Kitasato,[37] Carl,[38] Silberschmidt,[39] etc.) who indentified anaerobes which they had isolated from various sources with the malignant edema bacillus of Koch, state that the bacillus peptonizes the coagulated milk and produces in this medium a very disagreeable odor. On the other hand, the statements regarding the "vibrion septique" are incomplete. Arloing[40] mentions that the virus of septicemic gangrene ferments corbohydrates and albuminoids; Besson,[41] on the other hand, states in his text-book that the "vibrion septique" peptonizes coagulated egg albumen, serum, etc. The organism described by the writer never showed peptonization in any medium. The study of the literature gave the impression at first that the bacillus isolated from the hogs represented a new anaerobe. However, by studying carefully the splendid book and identification tables of v. Hibler,[42] the writer found a proper identification immediately possible. The bacillus corresponds in every detail with the so-called Ghon-Sachs bacillus.[43]

The Ghon-Sachs bacillus does not blacken the brain medium, or change its reaction to alkaline; the malignant edema bacillus, however,

32. Mitt. a. d. k. Gsndhtsamte, 1881, 1, p. 49.
33. Ibid., p. 93.
34. Bull. de l'Acad. de méd., 1877, 6, p. 781. Compt. rend. Acad. d. sc., 1878, 86, p. 1037. Bull. de l'Acad. de méd., 1881, 10, pp. 78, 81, 142.
35. Ztschr. f. Hyg. u. Infektions-krankh., 1886, 1, p. 158.
36. Deutsch. Ztschr. f. Tiermedizin, 1888, 13, p. 41.
37. Ztschr. f. Hyg. u. Infections-krankh., 1889, 6, p. 107.
38. Deutsch. tierarztl. Wchnschr., 1895, 5, p. 115.
39. Korrespondenzbl. f. Schweizerärzte, 1900, 30, p. 361.
40. Leçons sur la tuberculose et certaines septicémies, 1892.
41. Technique microbiologique et sérothérapique, 1911, p. 288. Untersuchungen über die pathogenen Anaeroben, etc., 1908.
42. Berichte d. naturwissenschaftl. medizinsch. Vereins in Innsbruck, 1908-1909.
43. Centralbl. f. Bakteriol., I, O., 1903, 34, p. 301; 1904, 35, p. 655; 1904, 36, p. 1.

does. The milk when coagulated is not peptonized, and remains acid when inoculated with the Ghon-Sachs bacillus. The spores are resistant to a temperature of 99 C. for 8 minutes only. On the other hand, the spores of the malignant edema bacillus resist this temperature for more than 60 minutes. Furthermore, the serologic tests permit a distinct separation of these organisms.

The description given by Ghon and Sachs[43] of the bacillus isolated by them in 1901 and reported in 1903, corresponds in every respect with the bacillus isolated from our hogs. These two writers have already indicated that their organism was identical with the "vibrion septique"; but, inasmuch as no complete description of the true malignant edema bacillus (Koch) was on hand, the bacillus isolated by Ghon and Sachs, the "vibrion septique," and the malignant edema bacillus were thrown together and identified as one and the same bacterium.

Through the careful comparative study of v. Hibler,[44] a separation of the organisms has now been accomplished, and any future work should first be compared with his very detailed description of the various pathogenic anaerobes.

The Ghon-Sachs bacillus has been recognized and properly identified 7 times in diseased conditions, all of which resembled, clinically, malignant edema or gas-phlegmon—1 case in man reported by Ghon-Sachs,[43] 4 cases by v. Hibler,[44] 2 in cattle (v. Hibler[45]), and 1 in a horse (Schlemmer[46]).

In animal pathology it unquestionably plays a considerable rôle. The various descriptions given by Miessner,[47] Markoff,[29] Levens,[48] and others for anaerobic organisms responsible for parturient symptomatic anthrax, bradsot of sheep, and blackleg of horses, correspond in almost every point with the facts brought forward by this study. Most of the workers, however, did not extend their studies sufficiently far to enable them to identify the organisms with the Ghon-Sachs bacillus.

The organisms isolated from hogs by Mareck,[1] even tho morphologically identical with B. chauvæi, had a high pathogenicity for rabbits; the publications of Mareck do not contain any detailed account as to the biochemical actions of the observed organism. The same remarks apply to the publications of Born[2] and Battistini[3] in which,

44. Untersuchungen über die pathogenen Anaeroben, etc., 1908, p. 2.
45. Ibid., 1, p. 404, p. 405.
46. Berl. tierärztl. Wchnschr., 1913, 19, p. 905.
47. Mitt. a d. k. Wilhelm-Inst. f. Landwirtsch. in Bromberg, 1909, 1, p. 28.
48. Berl. tierärztl. Wchnschr., 1911, 17, p. 413, p. 673.

based purely on morphologic similarity of the observed organisms in the diseased muscles, the diagnosis of symptomatic anthrax in hogs was made. Kitt[49] observed blackleg-like lesions in several wild hogs and, as a causative agent, he stated that he found the bacillus of malignant edema. Von Hibler isolated the "Novy bacillus" from the dried muscle of a wild hog which had succumbed to a blackleg-like infection following a lacerated wound on the hind leg.

The study of the anaerobe had already been completed when, in September, 1914, I found a reference to a publication by Köves[50] in which the same disease in hogs under discussion in this paper had been investigated on about 15 hogs. Later, I found that in February, 1914, Köves[51] summarized, in a preliminary note, his work on so-called "Rauschbrand der Schweine," stating that he found the Ghon-Sachs bacillus to be responsible for the affection. Furthermore, he mentions that in some cases of extensive diphtheric gastritis in hogs, which has always been considered as typical for hog-cholera, he isolated from the serosa of the stomach the Ghon-Sachs bacillus. The identity of the organism was proved by animal inoculations; the organism isolated from the serosa of the stomach also produced typical muscle lesions with gas formation. The conclusion that the so-called "symptomatic anthrax" of swine and the bradsot-like affections of these animals are etiologically an entity, is now fully proved by my independent investigations.

In the introduction to this paper I stated that "symptomatic anthrax" in swine had not been observed in the United States; the same statement also includes infections due to the Ghon-Sachs bacillus. All the strains obtained from the American Museum of Natural History behaved like the B. œdematis maligni (Koch) and a careful review of the existing literature indicates that all organisms isolated from cases of malignant edema peptonize the milk and coagulate proteins. How carefully such studies have been carried out is not always evident. One statement, made by Sperry and Rettger,[52] that the bacillus of symptomatic anthrax causes rapid decomposition of fibrin, egg, and meat mixtures, and reduction of solid matter, suggests in the light of all of the comparative work on B. chauvæi published in the international literature, that the strains used in some laboratories

49. Bakterienkunde und pathologische Mikroscopie, 1908, p. 290.
50. Deutsch. tierärztl. Wchnschr., 1914, 22, p. 536.
51. Berl. tierarztl. Wchnschr., 1914, 30, p. 134.
52. Jour. Biol. Chem., 1915, 20, p. 455; 1906-07, 2, p. 79.

have not always been properly identified. It would be very interesting if careful data as to the frequency and occurrence of the Ghon-Sachs bacillus in the United States were collected.

In a recent report on studies on hog-cholera, Dinwiddie[53] describes lesions in the stomach of hogs, and an anaerobic organism, the first being similar to those noted and described by Köves, the latter probably identical with the strains isolated by myself and therefore belonging to the Ghon-Sachs bacillus type. The intestinal lesions, in form of a severe diphtheric gastritis described by Köves as being caused by the Ghon-Sachs bacillus, are very frequently seen and are unquestionably mistaken for hog-cholera. An extensive investigation, guided by these stated facts, would therefore be a very gratifying task.

The organism seems to be widely distributed, not confined to Europe and America only; a description given from Australia by Gilruth[54] on a variety of the "vibrion septique" non-pathogenic for rabbits corresponds in many respects with the Ghon-Sachs bacillus.

The discussion cannot be closed without considering the experiments by v. Ratz[8] regarding the transmissibility of symptomatic anthrax to hogs. In 9 experiments, in which primary muscle-juice of cattle or hogs was used, v. Ratz succeeded in infecting 9 animals, of which 3 died. Experiment 2 consisted in the inoculation of a hog with muscle juice from so-called "symptomatic anthrax" in a hog. The lesions resembled strongly those described in this paper and, in the writer's mind, there is no doubt that v. Ratz was working, in this case, with the Ghon-Sachs bacillus. In two other successful experiments (4 and 8), muscle juice from cattle was used, and the changes resembled more those of "symptomatic anthrax." In no instance, however, did v. Ratz carry out cultural tests, nor does he report the methods he used to identify the organism he inoculated with the muscle juice. He injected the material from cattle, supposing that all lesions like symptomatic anthrax are caused by B. chauvæi. That his supposition is totally erroneous has been shown by Wulff,[30] Foth,[18] and many others. It is therefore natural to conclude that either the muscle material did not contain B. chauvæi, but the Ghon-Sachs bacillus; or a mixed infection of B. chauvæi with the Ghon-Sachs bacillus existed. In any case, these experiments do not prove that

53. Bull. Arkansas Exper. Station, 117, p. 600.
54. Veterinary Jour., 1911, 67, p. 471.

the hog has only a limited immunity to blackleg and that spontaneous cases are probably due to B. chauvæi.

The experiments of Glässner[6] and Wulff,[7] who attempted to infect hogs with blackleg material, are more careful. In 2 experiments the writer has inoculated small hogs with muscle material containing a very virulent B. chauvæi strain; aside from a slight, local swelling no symptoms were noted. I cannot therefore agree with v. Ratz that hogs are susceptible to symptomatic anthrax. Anatomically similar affections of hogs are probably always caused by the Ghon-Sachs bacillus which, morphologically, is so remarkably similar to B. chauvæi.

CONCLUSIONS

The report describes the recognition in the United States of a disease of hogs, well described in European publications, which anatomically resembles symptomatic anthrax in cattle. The cases studied belonged to a small epidemic and are not sporadic cases as reported from Hungary.

The lesions and the deaths of the animals were due to the Ghon-Sachs bacillus, which was properly identified by all well-known means. Etiologically, the American cases are therefore identical with the European ones.

The methods and media recommended by v. Hibler, the agglutination tests and the serum immunization of guinea-pigs, proved to be very reliable for the separation and identification of closely allied anaerobes.

The name of "specific gas phlegmon of hogs" is proposed by the writer for the disease.

The study of this disease, additional experiments, and a critical survey of the literature fail to prove that hogs are spontaneously attacked by symptomatic anthrax, or that they are susceptible to B. chauvæi.